

Original Article

A comparative study of blood culture and immunochromatographic assay for rapid diagnosis of typhoid fever

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Abstract

Background and objectives: Typhoid fever, caused by *Salmonella* Typhi, is an important cause of morbidity and mortality in many developing countries including Bangladesh. As early diagnosis of disease and prompt treatment are essential for optimal management, a rapid method is urgently needed. Blood culture is the gold standard for diagnosis of typhoid fever, but it is time consuming. Immunochromatographic test (ICT) is easily accessible, cheap and simple method for diagnosis of typhoid fever, especially in the resource poor countries like Bangladesh.

Methodology: 180 samples were collected from suspected typhoid fever cases. This study was conducted to isolate the *S. Typhi* by blood culture and to detect IgM and IgG antibody to *S. Typhi* by ICT method.

Results: Out of 180 samples, 105(58.33%) were positive in ICT and 24(13.33%) were positive in blood culture. Considering blood culture as gold standard, the sensitivity, specificity, positive predictive value of ICT was 91.67%, 46.79%, 20.95% and 97.33% respectively.

Introduction

Typhoid fever is a major cause of death worldwide with a major part of the disease burden in developing regions such as the Indian subcontinent¹. The highest disease burden of typhoid fever caused by *Salmonella* Typhi is seen in young children. The relative risk for children compared with older persons was 8.9². Since all the sign symptoms of typhoid fever are non-specific, a definitive diagnosis of the disease depending on the clinical presentation alone is difficult. Therefore, laboratory-based investigation are essential for supporting the diagnosis of typhoid fever. Blood culture is the Gold standard method of diagnosis of typhoid fever³. Rapid and sensitive laboratory method for diagnosis of typhoid fever is essential for prompt and effective therapy. An early diagnosis and prompt treatment are mandatory not only for diagnosis but also for prevention of the spread of the organism by chronic carriers to the

community. However, Blood culture is insufficiently sensitive, technically demanding and bone marrow culture although more sensitive, is infrequently performed⁴. Organisms can usually be detected in 75-90% of patients during the first ten days of infection⁵. Blood culture is 100% specific for diagnosis of typhoid fever⁶. Therefore, a fast, reliable and easy to perform serodiagnostic test with a higher sensitivity and specificity than Widal test is required for diagnosis of typhoid fever⁷. The rapid immunodiagnosis of typhoid fever can be done by the detection of anti-salmonella antibodies by immunochromatographic test which does not require any specialized laboratory or highly skilled personnel and can be done in field areas also. It is a simple and rapid diagnostic test. In ICT, *S. Typhi* specific antihuman immunoglobulin against IgG and IgM fixed in the strip can detect both IgM and IgG anti-salmonella antibodies⁸. The test simultaneously detects and differentiates the IgG and IgM antibodies to *S. Typhi* specific antigen in whole blood or serum⁹.

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Rapid and sensitive laboratory method for diagnosis of typhoid fever is essential for prompt and effective therapy. Keeping these areas of concern in mind, this study has been designed to compare conventional blood culture with serological test (ICT) for the rapid and reliable diagnosis of typhoid fever.

Materials and Method

This cross sectional study was carried out at Department of Microbiology, Chattogram Medical College, Chattogram, Bangladesh over a period of one year from July 2018 to June 2019.

A total of 180 blood samples from patients clinically suspected to have typhoid fever were collected for both culture and serological test.

The blood culture was performed by inoculation of 30 ml of trypticase soya broth with 3 ml blood in children and 50 ml of trypticase soya broth with 5 ml of freshly collected blood in adult. The bottles were examined visually daily. Growth was indicated by haemolysis of red blood cells, gas bubbles in the medium or turbidity in the broth. In addition to daily visual examination, blind subculture from conventional bottle after the first 24 hours of incubation was performed by aseptically removing a few drops of the well-mixed medium and spreading the inoculums into MacConkey agar and Blood agar plates. Plates were incubated at 37°C aerobically for 24-48 hours. Culture negative bottles were reincubated for 5 to 7 days⁵. Presumptive identification of the colonies of *S. Typhi* on MacConkey agar media and Blood agar media were done by colony morphology, oxidase test and Gram staining from growth. *S. Typhi* was confirmatively identified by using triple sugar iron (TSI) medium, citrate utilization test and test on MIU media¹⁰.

The immunochromatographic test was carried out as manufacturer's instruction (Acro, Biotech, INC, USA). 1 drop of serum was put to the sample well of the test unit, then 1 drop of sample diluents added immediately. The test unit was kept for 15 minutes. Results were read after 15 minutes. Positive result was visible within 1 minute.

Results

From the 180 clinically suspected cases, 108 (60%) were male and 72 (40%) were female. Out of 180 clinically diagnosed cases, 24 (13.33%) cases were blood culture positive for *Salmonella Typhi* and the remaining

156(86.67%) cases were negative. Among culture positive cases, most cases belongs to 6-10 years of age which is 25% followed by 20.56% cases belonging to 1-5 years of age. Of 180 suspected cases of typhoid fever, ICT was positive by 105 (58.33%) cases whereas only IgM were positive for 76 cases and both IgM and IgG were positive by 29 cases. Here, only IgM positive and both IgM and IgG positive were considered as positive.

To calculate the diagnostic validity of ICT for the diagnosis of typhoid fever taking blood culture as a gold standard, it was found that, of 180 cases, 22 cases were positive by both blood culture and ICT whereas, out of 156 culture negative cases, 83 (46.11%) cases were only positive by ICT.

In this study, 22(12.22%) cases were true positive and 02(1.11%) were found false negative.

Here, out of 180 cases, 73(40.56%) clinically suspected typhoid fever cases found were both blood culture and ICT negative.

After calculation it was found that the sensitivity, specificity, positive predictive value and negative predictive value of ICT were 91.67%, 46.79%, 20.95% and 97.33% respectively.

Table 1: Diagnostic validity of ICT

Test results	ICT		Sensitivity	Specificity	PPV	NPV
	Positive	Negative				
Culture positive =24(13.33)	22(12.22)	02(1.11)	91.67%	46.79%	20.95%	97.33%
Culture negative =156(86.67)	83(46.11)	73(40.56)				
Total = 180(100)	105(58.33)	75(41.67)				

Figures in parenthesis indicate percentage (p value=0.00003743, p <0.05 by chi-square test)

Discussion

Typhoid fever is an acute systemic infection caused by the bacterium *Salmonella enterica* serovar Typhi and a human host restricted organism¹¹. Typhoid fever is transmitted by the feco-oral route via the contaminated food and water and is therefore common where sanitary conditions are inadequate and access to clean water is limited¹². Although, blood culture is the Gold Standard for diagnosis of typhoid fever, but isolation of the organism by blood culture is quite time consuming and blood culture facility

are not well established in most of the areas. On the other hand, ICT is simple, rapid and easy to perform for diagnosis of typhoid fever.

In the present study, 180 suspected cases of typhoid fever of all age of both sexes irrespective of antibiotic therapy were enrolled for the detection of typhoid fever. Of them, 24(13.33%) cases were found positive for *S. Typhi*. Similar findings were also reported from India, 14% blood culture positive¹³ and 12.22% culture positive¹⁴. The relative low rate of isolation from blood may be because of factors such as the bacteriostatic effect of antimicrobials which were already administered to some patients before blood culture was done, the time of sample collection, the type of culture medium used, the host immune response and the intracellular characteristics of *S. Typhi*¹⁵. In contrast to our study, 24 (57.14%) culture positive cases among 42 suspected typhoid fever cases were found¹⁶. Differences may be due to small sample size.

In present study, 105 (58.33%) cases were found positive for ICT among 180 clinically suspected cases of typhoid fever where we considered only IgM positive and both IgM and IgG positive as positive. Similar study from Pakistan was observed where 55.2% were positive by ICT¹⁷. In contrast to our study, another study from Bangladesh found only 24.7%¹⁸.

In our study, among 24 culture positive cases, 22 (91.67%) were positive by ICT. Another study from Bangladesh found 54 (93.1%) ICT positive cases out of 58 culture positive cases¹⁹. ICT negative result in culture positive cases was probably due to the fact that antibodies did not yet reach the detectable level.

In our study, considering blood culture as “**Gold Standard**” the sensitivity, specificity, positive predictive value and negative predictive value of ICT were 91.67%, 46.79%, 20.95% and 97.33%. Another study carried out in India reported that the sensitivity, specificity, positive predictive value and negative predictive value of ICT were 96.43%, 54.4%, 25.96% and 98.92%²⁰.

It is therefore, concluded that the ICT method is superior to blood cultures yielding very high sensitivity. Detection of antibody from whole blood by ICT method is easier, simple and non-invasive. ICT method is useful for early initiation of treatment. It should be adopted in routine clinical investigation for rapid diagnosis of typhoid fever in resource poor settings.

Acknowledgements

This study was partially funded by DGHS, Bangladesh under Issue No: DGHS: ME& HMD/2018-19/241, Issue date: 25-04-2019.

Conflict of interest

There is no conflict of interest.

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